Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

NOTES ON *NOSEMA APIS* ZANDER*

R. Kudo

Aside from *Nosema bombycis* Nägeli, no other Microsporidian has received so much attention of investigators as *Nosema apis* Zander. A disease of adult honey bees for which the Microsporidian is responsible, and which is known by different names such as Nosema-Seuche, Isle of Wight disease, Nosema disease, etc., has been reported to occur in various parts of the world. In North America, White (1914) found 40 infected bees in 120 samples received from 27 states of the union, and two diseased samples received from Canada. The same author further published (White, 1919) valuable results of experiments on the means by which the spores may be killed.

The morphology and development of the Microsporidian have, however, been studied by but three investigators, i. e., Zander (1911) and Fantham and Porter (1912). According to these authors, the spores of *Nosema apis* are on the whole similarly constructed to those of *Nosema bombycis* studied by Stempell (1909). Recently, I had an opportunity of studying the Microsporidian, and have obtained more or less different results by observations upon the structure of the spore from those of the above mentioned European investigators. I shall briefly describe the results in the following pages.

In a field at Spring Valley, New York, 660 workers of *Apis millifica* were collected from August 27 to September 5, 1920. Although I could not trace the hive for these captured bees, there were a number of hives in the nearby woods where the collection was made. The digestive tract of each bee was drawn out from the body by means of a pair of forceps and a needle. A part of the ventriculus was smeared on a slide and examined microscopically in the fresh state.

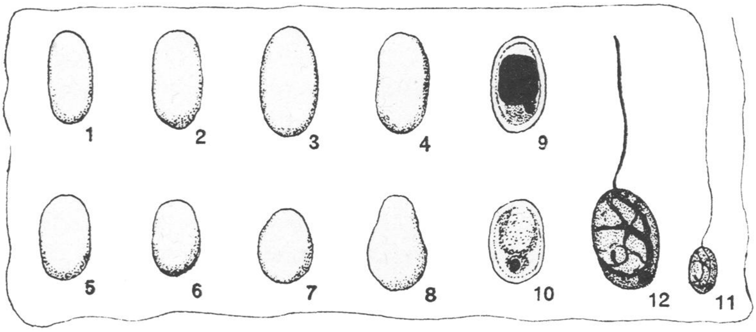
Out of 660 bees thus examined, 25 were found to be infected by *Nosema apis*. After encountering one or two heavily infected bees, it was not difficult to diagnose a heavily infected host insect by its inactivity and peculiarly softened abdomen. When a part of a heavily infected ventriculus was placed on a slide, the milky white appearance gave a definite sign of the nature of the infection before a microscopical examination was made. Four bees contained spores similar to those of *Nosema apis*, differing, however, in larger dimensions and a large clear rounded space at one extremity. The presence of a

---

* Contribution from the Zoological Laboratory of the University of Illinois, No. 167.

space which is generally called a vacuole, in the mature spores, recognizable in fresh state, has commonly been noted in many species of Microsporidia. Frequently the spores of *Nosema apis* show also a clear rounded area near at one end, which, however, does not occur so regularly as in the spores from the above mentioned four bees. Whether this form should be distinguished from *Nosema apis* by a different specific name or not will have to be determined by a comparative study on the various stages of their development.

The dimensions of spores of *Nosema apis* differ somewhat widely according to different authors. According to Zander (1911), the spore meausres 5μ in length and 2.86μ in breadth, and the polar filament seemed to be shorter than that of *Nosema bombycis* measured by Stempell (1909). Fantham and Porter (1912) recorded the length and breadth 4 to 6μ (up to 7μ) and 2 to 4μ, respectively, and stated that the polar filament was "about 60μ" long. White's measurements (1919) are as follows: fresh spores in India ink smears, 4.46μ long by 2.44μ broad; stained spores, 4.15μ long by 2.06μ broad.



Spores of *Nosema apis*. Figs. 1-8. Fresh spores of various form and size. × 2350. Fig. 9. A spore stained with Heidenhain's iron hematoxylin. × 2350. Fig. 10. A spore stained with Giemsa's solution. ×2350. Fig. 11. A spore with an extruded polar filament, stained after Fontana (the polar filament is diagrammatically shown without changing its approximate length). × 1200. Fig. 12. A more highly magnified view of the spore shown in fig. 11. ×2350.

According to my observations, fresh mature spores vary from 4.6 to 6.4μ in length, and from 2.5 to 3.4μ in breadth and thickness. The dimensions were obtained by measuring 250 spores. As mature spores (Figs. 1-8) differ to a more or less great degree in form and size, care has been taken in choosing the spores. I have measured ten spores from a smear of each infected individual, one spore taken at random near the center in every other field.

In the fresh condition, a spore does not show any differentiation in its contents. It is slightly less refractive than that of *Nosema bombycis*. The polar filament is invisible in fresh state, as in the majority of microsporidian spores, except such a form as *Thelohania magna* (Kudo, 1920). The presence of a polar filament in the spore, therefore, could only be proved by causing its extrusion. Zander (1911) made no effort, and mentioned simply that it seemed to be shorter than that of *Nosema bombycis* as studied by Stempell. Fantham and Porter (1912) stated that it measured about 60μ in some spores they had examined.

The polar filament of *Nosema apis* can easily be extruded and observed, although some authors are inclined to think it is "very difficult of observation under any circumstances" (Fantham and Porter, 1912: 174). Either mechanical pressure (Kudo, 1913) or perhydrol (Kudo, 1918) causes extrusion of the filament in fresh spores. For quick observation a dark field microscope is indispensable (Kudo, 1918). But for permanent preparation, Fontana's staining (Kudo 1920) gives best results according to my more recent observations.

As to the method of application of mechanical pressure upon the spores, I stated briefly (Kudo, 1913) that they were pressed by fingers between a cover glass and a slide. Since I have often been asked as to the exact technique, I shall briefly describe the method here. A very small drop of water emulsion of fresh microsporidian spores is placed on a slide by means of a fine capillary pipette and is covered with a cover glass. It is desirable to have the outer margin of the cover glass unoccupied by the emulsion. Place the slide on a smooth and steady surface, and cover the cover glass with a piece of cloth or filter paper, over which the elbow is gently applied. Give a strong downward push to the arm. This will instantly cause the extrusion of polar filaments. The slide now can be brought on the stage of a dark field microscope and examined. To make the preparation permanent the cover glass must be lifted up carefully. After being fixed with fixative such as sublimate alcohol, the smear is stained after Fontana.

The completely extruded polar filament of spores of *Nosema apis* measures from 230 to 280μ in length. The remarkable difference in the length of polar filaments compared with that obtained by Fantham and Porter, is due solely, I believe, to the difference in the technique applied. In the case of *Nosema bombycis,* I have already shown (Kudo, 1913, 1916) how different methods bring out entirely different results regarding this delicate structure characteristic of Microsporidia. Iodine water has been used by several investigators, including the above mentioned English authors, in causing the filament extrusion of microsporidian spores. Yet evidences obtained by comparative studies of various methods have shown me that the iodine water in this case is not likely to cause the extrusion, but simply stains the already extruded polar filaments, most probably due to the pressure of the cover glass, to a more or less recognizable extent, showing, therefore, only incompletely extruded polar filaments in very small numbers.

When examined under a dark field microscope (Fig. 13), the extruded filament of *Nosema apis* shows a uniform thickness throughout, as those of other Microsporidia which I have studied up to the present. Its form is, however, very striking in some cases. Leaving the spore at one end, it shows about from 10 to 15 undulating courses of a large wave length. This part is followed by another about 10 to 15 turns of uniformly small wave length. Somewhat similar conditions were frequently noticed in the polar filament of *Nosema bombycis,* extruded under the influence of perhydrol (Kudo, 1918, Fig. 5), but it was not so conspicuous as in the present Microsporidian. As far as I am aware, this peculiar condition has never been reported to occur. This wavy form becomes straightened out gradually and loses its former appearance, although the distal portion often remains in more wavy condition than the basal. Some polar filaments are, however, as straight as a stretched thread. The difference in the form of the extruded filament is due, in my opinion, to the difference in pressure received. It seems probable that when the spore receives a sudden violent pressure, the polar filament escapes from the spore without unwinding its coiled form, and that when the spore receives a gradually increasing pressure, the extrusion of the filament takes place slowly in a more or less straightened form. When the cover glass is removed from the slide, the agitation of the emulsion in which the spore is floating, apparently tends to straighten the filament, so that after Fontana's staining straight forms tangled in various ways are mostly to be found. Fontana's method stains not only the extruded polar filament, but also its unextruded portion. In this regard, the method has superiority over Löffler's (Kudo, 1913). Thus in the spore shown in Figs. 11 and 12, the unextruded part of the filament is clearly distinguishable, suggesting the extrusion in this particular spore was incomplete.

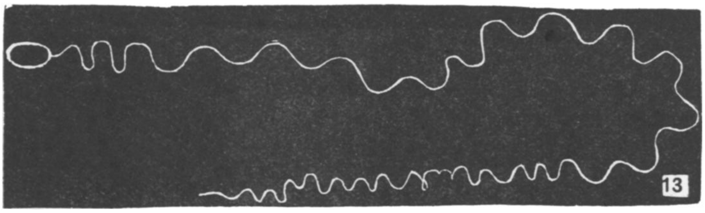


Fig. 13. A spore of *Nosema apis* with the extruded polar filament viewed under a dark field microscope. ×1200.

When the spores are fixed and stained, the contents appear more or less differentiated. In spores stained with Giemsa's solution, a small rounded sporoplasm taking a typical blue color with its deeply red nucleus, is seen near one end, while between this and the other end of the spore, the polar capsule with its polar filament is observable (Fig. 10). In spores stained with Heidenhain's iron hematoxylin, similar differentiation appears, although the polar capsule with its filament frequently stains as deeply as the nucleus of the sporoplasm (Fig. 9). The polar filament does not start to coil close to the end of the spore, to which it is attached. The basal portion is rather straight and begins to coil some distance back (Figs. 9 and 14). Zander (1911) observed this portion, but misinterpreted the relative position of the polar filament and the sporoplasm. Thus the structure of the spore of *Nosema apis* is similar to that of *Thelohania magna* (Kudo, 1920).

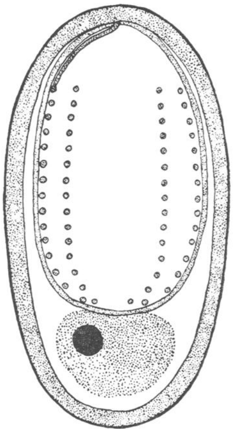


Fig. 14. A schematic representation of the longitudinal section through a mature spore of *Nosema apis*. Except for the basal portion the doubly coiled polar filament appears only in cross sections. ×10,000.

The fact that the polar filament of the spore of *Nosema apis* when extruded shows under certain circumstances two regions, one with a regularly large wave length, and the other with a uniformly small wave length, each having 10 to 15 turns, leads me to assume that the mode of coiling of the filament inside of the spore is a particular one. The only interpretation that can be advanced in this connection is that the polar filament of the spore of *Nosema apis* is coiled from 10 to 15 times along the polar capsule, inside of which and continuous to it, it is coiled back again toward the tip where the filament is attached. To illustrate the structure, a schematic longitudinal section of a spore is shown in figure 14.

SUMMARY

1. Among honey bees collected at Spring Valley, New York, from August 27 to September 5, 1920, 3.8 per cent. were found to be infected by *Nosema apis*.

2. Four bees harbored an undetermined Microsporidian.

3. Fresh spores of *Nosema apis* measure from 4.6 to 6.4μ long, and from 2.5 to 3.4μ broad and thick.

4. A method of applying mechanical pressure to cause the filament extrusion of microsporidian spores, is described.

5. The polar filament of the spore of *Nosema apis* is 230 to 280μ in length. The structure of the spore is similar to that of *Thelohania magna*.

6. Extruded polar filaments show two parts, one with larger and the other with smaller undulations, each composed of 10 to 15 turns, indicating that the filament is doubly coiled.

PAPERS CITED

Fantham, H. B., and Porter, Annie. 1912.—The morphology and life history of *Nosema apis* and the significance of its various stages in the so-called "Isle of Wight" Disease in bees (Microsporidiosis). Ann. Trop. Med. Parasit., 6: 163-195, 3 pl. and 1 textfig.

Kudo, R. 1913.—Eine neue Methode die Sporen von *Nosema bombycis* Nägeli mit ihren ausgeschnellten Polfäden dauerhaft zu präparieren und deren Länge genauer zu bestimmen. Zool. Anz., 41: 368-371, 4 fig.

1916.—Contributions to the study of parasitic protozoa. I. On the structure and life history of *Nosema bombycis* Nägeli. Bull. Imp. Seric. Exp. Stat. Tokio, 1: 31-51, 2 pl.

1918.—Experiments on the extrusion of polar filaments of Cnidosporidian spores. Jour. Parasit., 4: 141-147, 1 pl.

1920.—On the structure of some Microsporidian spores. Jour. Parasit., 6: 178-182, 11 fig.

White, G. F. 1914.—Destruction of germs of infectious bee diseases by heating. Bull. U. S. Dept. Agr., no. 92, 8 pp.

1919.—Nosema Diseases. Bull. U. S. Dept. Agr., no. 780, 59 pp., 4 pl. and 7 figs.

Zander, E. 1911.—Handbuch der Bienenkunde. II. Krankheiten und Schädlinge der erwachsenen Bienen. Stuttgart. 42 pp., 8 pl. and 13 figs.